PREVALENCE AND ANTIMICROBIAL RESISTANCE PROFILES OF *Escherichia coli* ISOLATES FROM BROILER AND LAYER CHICKENS IN ARUSHA AND MWANZA, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN EPIDEMIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

2021

ABSTRACT

Microorganisms of clinical importance have developed resistance to currently used antimicrobials. The spread and increased reports of antimicrobial-resistant pathogens such as *Escherichia coli* is one of the important dynamics lessening treatment and prophylaxis possibilities; thus posing a threat to modern human medicine, veterinary medicine and food safety. Therefore, this study aimed at determining the prevalence of antimicrobial resistance (AMR) in *E. coli* isolates obtained from broiler and layer chickens in Mwanza and Arusha regions in Tanzania. A cross-sectional study was carried out from February to March 2021 in 402 poultry farms in Mwanza (201) and Arusha (201) regions in Tanzania. *E. coli* was isolated from all 402 samples by culture and was confirmed using MALDI-TOF. Two hundred and four (204) *E. coli* isolates were randomly chosen and antimicrobial susceptibility testing was performed using the disc diffusion method. Isolates were tested against seven antimicrobial agents belonging to seven classes of antimicrobials and results interpreted using CLSI standards. Data were entered in Microsoft Excel® and descriptive statistics were computed to obtain the prevalence using SPSS version 20 and the *P*-value was set at 0.05. All the tested isolates (n=204) were resistant to at least one antimicrobial agent. Overall, the highest and lowest AMR prevalence was observed for ampicillin (100%) and gentamicin (10.3%), respectively. The majority of the isolates (86.76%) were multi-drug resistant. The AMR of *E. coli* to four classes of antimicrobial agents was the highest in this study (31.1%). Only six (2.9%) isolates were resistant to all the seven classes of antimicrobial agents. The prevalence of extended-spectrum beta-lactamase (ESBL) producers was 10.29% with all the isolates (n=21) expressing bla_{TEM} genes and only two isolates (2/21) expressing $bla_{\text{CTX-M}}$ .The isolates obtained in this study displayed high antimicrobial resistance to commonly used antimicrobial agents in veterinary and human medicine. This implies that there might be

some practices that accelerate antimicrobial resistance in the production of the sampled birds. Integration of appropriate use of antimicrobial agents and other measures to curb the spread of resistant genes is necessary.

DECLARATION

I, Ruth Wairimu Kiiti, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor currently being submitted for a higher degree in any other institution

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ACKNOWLEDGEMENTS

I would like to express special thanks to God for His divine guidance and providence. Thanks are due to my supportive supervisors; Prof. P. L. M. Msoffe and Prof E.V.G. Komba who have tirelessly, kind-heartedly, sacrificed, and contributed a lot to the development of this work.

I would like to thank Ms. R. Nshama, Mr. E. Oswald, Mr. R. Mikidadi, Ms. S. Esther, Ms. A. Igizeneza, Ms. F. Ngwewa, Ms. M. Byonaneye, Mr. P. Mkuchu, Dr. E. Mwenga, and Dr. A. Mzula for the technical support they offered to me during the laboratory analysis of samples. I extend grateful acknowledgement to my family for the great support and love they have offered to me.

DEDICATION

With great pleasure, I am dedicating this work to my lovely nephew (Kiiti Sila) and nieces (Patience Ngonyo and Annah Mbinya).

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LIST OF ABBREVIATION

AMP	Ampicillin
AMR	Antimicrobial resistance
C	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical laboratory standard institute
CN	Gentamycin
CRO	Ceftriaxone
ESBL	Extended Spectrum β-Lactamase
ETP	Ertapenem
STX	Trimethoprim/Sulfamethoxazole
WHO	World Health organization

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background information

Escherichia coli is a commensal bacterium, however, some of its strains have developed a potential to cause intestinal and extra-intestinal infections (Vila *et al*., 2016); and have also developed resistance to commonly used antimicrobial agents (Olorunmola *et al*., 2013). These disease-causing and resistant strains of *Escherichia coli* pose a big risk to human and animal health as well as food safety in case they develop antimicrobial resistance mechanisms (WHO, 2017).

Antimicrobial resistance (AMR) is a state whereby microbial pathogens are no longer affected by antimicrobials by developing mechanisms towards evading antimicrobial drugs (WHO, 2015; WHO, 2017). The process is accelerated by the influx of Antimicrobial Resistance Genes (ARGs) to the environment from livestock and human wastes and by the vast quantities of antimicrobial residues discharged from the pharmaceutical industry, hospitals, and intensive livestock farms (O'Neill, 2014; Robinson *et al*., 2016; Sumanth *et al.*, 2017). Approximately 4.2 million deaths occur annually in Africa due to antimicrobial resistance, and 300 million people are at risk of dying prematurely worldwide due to drug resistance over the next 35 years (O'Neill, 2014). The global GDP is expected to drop by 2 to 3.5% by 2050, which is translated into losing 60 to 100 trillion USD if the AMR issue will not be tackled adequately (O'Neill, 2014). Antimicrobial drugs have increasingly been applied or administered as preventive chemotherapy (Sattar *et al*., 2014; Lammie and Hughes, 2016) and growth promoters (Ezenduka *et al*., 2014) in livestock production industries such as dairy farms, poultry farms, and pig farms as well as many other industries globally. Globally, the

antimicrobial use in animals is estimated to increase by 67%, (from 63, 151 to 105, 596 tonnes), between 2010 and 2030 as a result of increased use of foods of animal origin and more intensive animal farming practices in middle-income countries (Van Boeckel *et al*., 2015).

In Tanzania, AMR remains a growing problem in humans, animals, and the environment (Moremi *et al*., 2016; Katakweba *et al*., 2018; Subbiah *et al*., 2020). Through multiple research, antimicrobial resistance has been detected among isolates from chicken in the country (Nonga *et al*., 2010; Hamisi *et al*., 2014; Rugumisa *et al*., 2016; Mgaya *et al*., 2021; Kimera *et al*., 2021).

Tanzania has three types of production systems in the poultry sector: traditional indigenous, improved family chicken, and commercial specialized chicken systems (Da Silva *et al*., 2017). The population of chickens in the country is estimated at seventy-two million (forty million are indigenous and thirty-two million are exotic). Over 24 million are broilers and 8 million are layers (NBS, 2016; TLMP, 2018; Vernooij *et al*., 2018). At the end of their production cycle, layer chickens also enter the food chain as spent birds. This has implications on possibilities of widespread human health effects if these chickens are colonized with zoonotic pathogens especially when the pathogens are AMR. However, the extent of the AMR problem in the broilers and layers is not sufficiently known, partly because of the absence of regular surveillance and inadequate laboratory capacity and capability. In the absence of reliable data, mitigations against AMR are inadequately implemented.

This research aimed at determining the prevalence and antimicrobial resistance profiles of *Escherichia coli* isolate obtained from broiler and layer chicken in Tanzania. Data generated from this study will supplement the existing antimicrobial resistance profiles of *E. coli* isolated from broilers and layers in Tanzania.

1.2 Problem statement and study justification

Chicken production is an essential source of animal protein and income in Tanzania. The increased spread of antimicrobial-resistant pathogens such as resistant *E. coli* is a major factor limiting treatment and prophylaxis options, threatening modern human medicine, veterinary medicine, and food safety (WHO, 2017). Human activities have been reported to contribute to the evolution and spread of bacteria that have already developed resistance to antimicrobial agents in the environment (Pruden *et al*., 2012). Circumstantial evidence indicates that the farmers normally carry out treatment to poultry ailments using antimicrobials with little or no supervision from veterinarians leading to overuse and misuse of antimicrobial drugs (Karimuribo *et al*., 2005; Caudell *et al*., 2018; Kimera *et al*., 2020). The trend of antimicrobials use in the livestock sector in Tanzania is alarming (Hounmanou and Mdegela, 2017). This study, therefore, provides up-to-date information on antimicrobial resistance profiles of *E. coli* that will help decision-makers to understand new resistance threats in Tanzania as well as to help prioritize the actions to be taken as mitigation measures. This study sought to provide this useful information by addressing the following objectives.

1.3 Objective

1.3.1 General objective

To determine the prevalence and antimicrobial resistance profiles of *E. coli* isolates from broiler and layer chickens in Tanzania.

1.3.2 Specific objectives

1. To isolate *E. coli* from broilers and layers from Arusha and Mwanza in Tanzania
2. To determine the prevalence and antimicrobial resistance patterns of *E. coli* isolated from broiler and layer chickens from Arusha and Mwanza in Tanzania.
3. To assess the phenotypic and molecular characteristics of extended-spectrum beta-lactamase-producing *E. coli* isolates obtained from broiler and layer chickens in Arusha and Mwanza in Tanzania.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Escherichia coli

E. coli is a commensal bacterium but some of its strains have developed the potential to cause intestinal and extra-intestinal infections (Vila *et al*., 2016). The main strains that call for attention are the extended-spectrum β-lactamase (ESBL) producing strains. The pathogenic strains include; Diffusely adherent *Escherichia coli* (DAEC) which leads to persistent diarrhoea in infants (Mansan-Almeida *et al*., 2013), Enteroaggregative *Escherichia coli* (EAEC) which adheres to microvilli via fimbriae and induces high production of mucus causing biofilm which leads to inflammatory response and production of toxins (Kong *et al*., 2015), Enterohemorrhagic *Escherichia coli* (EHEC) which is the causative agent of food associated severe diarrhoea (Croxen *et al*., 2010; Page and Liles, 2013), Enteroinvasive *Escherichia coli* (EIEC) which causes dysentery as in watery diarrhea with pus, mucus and blood (Ud-Din and Wahid, 2014) and Enteropathogenic *Escherichia coli* (EPEC) which inhibits the transportation of sodium ion and chloride ion in the cell resulting in a subsequent release of water into the intestinal lumen (Wong Fok Lung *et al*., 2014).

The extra-intestinal pathogenic *Escherichia coli* strains are; Enterotoxigenic *E. coli* strain the causative agent of acute traveler's diarrhoea (Fleckenstein *et al*., 2010), and Uro-pathogenic *Escherichia coli* which is responsible for most uncomplicated urinary tract infections (Subashchandrabose and Mobley, 2015). Avian pathogenic *Escherichia coli* (APEC) is found in the lower digestive tract of poultry immediately after hatch (Ballou, 2016). The APEC causes respiratory and Systemic disease in poultry (Stacy *et al*., 2014) hence translating to economic loss to the poultry sector. Farms, where poultry is kept, have been identified as reservoirs for APEC (Ewers *et al*., 2009) which in turn

could mean that the APEC genes of the strain in the farm can be transmitted to the successive generation. The plasticity of the genome of *E. coli* makes it to have a wide diversity in its lifestyle (Tenaillon *et al*., 2010). Maslikowska *et al*. (2016) found out in their study that patients with the ESBL and CRE (Carbapenem-resistant Enterobacteriaceae) strains stay longer in the hospital and pay 1.5-2.5 more times costs compared to the normal costs. This is because the infections caused by the strains off-put the treatment administered to the patients which translates into increased morbidity and mortality (Guh *et al*., 2015; Adler *et al*., 2016).

2.2 Antimicrobial agents

Several selected antibiotics used for chemotherapy and prophylaxis in poultry farms are known to be very crucial in animal health in Tanzania and globally (WHO, 2017). These antimicrobials belong to different classes including tetracyclines (tetracycline, doxycycline), aminoglycosides (gentamicin, neomycin), lincosamides (lincomycin), amphenicols (chloramphenicol), fluoroquinolones (enrofloxacin, ciprofloxacin), sulfas (sulfamethoxazole), and beta-lactams (penicillin, ampicillin), macrolides (tylosin), sulphonamides/trimethoprim (sulfamethoxazole/trimethoprim) (Thome *et al*., 2019; Caudell *et al*., 2018).

2.3 Antimicrobial resistance in *E. coli*

Antimicrobial resistance in bacteria can occur as a result of several mechanisms including; target alteration (whereby the site of target by the antimicrobial agent changes due to mutation of the gene of the bacteria) (Banin *et al.*, 2017), decreased permeability (gram-negative bacteria can reduce the permeability to filter antimicrobial agents from penetrating their cell membrane) (Maria-Neto *et al*., 2015). Efflux increase where the bacterium produces toxic substances hence rendering the antimicrobial agent ineffective (Blair *et al*., 2014). This can occur through two types of biological pathways; vertical

gene transfer and horizontal gene transfer (Caniça *et al*., 2015). Antimicrobial resistance in *E. coli* is caused by various methods such as mutations that were observed in fluoroquinolones and by the acquisition of mobile genetic elements ARGs as in the case of penicillinases and ESBLs (Olorunmola *et al*., 2013). In the host environment, the commensal *E. coli* among the *Enterobacteriaceae* represents a predominant vehicle for the dissemination of resistance markers to pathogenic bacteria. Olorunmola and others were able to show that resistant strains could transfer resistance genes to susceptible *E. coli* strain in vitro (Olorunmola *et al*., 2013). The ability to colonize many hosts, attain resistance easily and survive outside the host making *E. coli* a reliable indicator for selection pressure by antimicrobial use and expected resistance in pathogens (Tadesse *et al*., 2012). Inappropriate use of antimicrobial agents to promote growth and for treatment in the animal sector in Tanzania has contributed considerably to the rise and spread of AMR in the livestock sector (Hounmanou and Mdegela, 2018). . High levels of antimicrobial residues have been reported in foods of animal origin in the country (Hounmanou and Mdegela, 2018).

2.4 Transmission of resistant *E. coli* to humans

Increased prevalence and transmission of *E. coli* variants have been associated with the unregulated use of antimicrobials in low and middle-income countries (Salinas *et al*., 2019). The contamination of the environment, carcasses, chicken meat, and surfaces in slaughter areas with Extra-pathogenic *E. coli* can cause transmission of EPEC to humans (if the contaminated meat is consumed by humans and through cross-contamination of non-meat items) (Belanger *et al*., 2011). Studies have shown some genetic similarities between *E. coli* isolates obtained from patients diagnosed with urinary tract infections, meningitis, and septicemia and those which are found in chicken and chicken products that are consumed by humans in Germany (Laube *et al*., 2013). This

information sends an alarm that the broilers and layers can act as reservoirs of *E. coli* strains that are pathogenic to humans (Vounba *et al*., 2019).

2.5 Risks of antimicrobial resistant bacteria in chicken production in Tanzania

Several drivers are linked to the exacerbated development and transmission of antimicrobial-resistant bacteria in chicken production in Tanzania. These drivers include; uncontrolled (over-dosage and under dosage) use of antimicrobial drugs both in humans and chicken, uncontrolled disposal of human and veterinary drugs, direct discharge of effluents from pharmaceutical industries into the river, availability and ease access of over the counter antimicrobial drugs for poultry, administration of human medicine to treat poultry, failure to adhere to good hygienic practices and other biosecurity measures, routine use of the drugs even when the chicken is not sick, and failure to observe drug withdrawal period (Karimuribo *et al*., 2005; Caudell *et al*., 2018; Hounmanou and Mdegela, 2018; Kimera *et al*., 2020).

2.6 Extended-spectrum beta-lactamases

Extended-spectrum beta-lactamases (ESBL) are enzymes released by *Enterobacteriaceae* (*E. coli*, *Klebsiella pneumoniae*). These enzymes can hydrolyze oxymino-beta-lactam antimicrobial agents (Adeolu *et al*., 2016). They exhibit resistance to antimicrobial agents belonging to antimicrobial classes such as fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole which may be chromosomally or plasmid-encoded (Shayanfar *et al*., 2010). The most predominant ESBL enzymes are grouped into the following types; CTX-M, SHV, and TEM (Peterson and Bonomo, 2005). *E. coli* strains carrying genes for various ESBLs including CTX-M have been reported in healthy humans, livestock, pets, food products, water, and sewage (Zheng *et al*., 2012; Jafri *et al*., 2014; Rasheed *et al*., 2014; Franz *et al*., 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted in Arusha and Mwanza regions. The regions are known to be high chicken producing (broilers and layers) areas in Tanzania with deviations in production existing between them. In these regions, the demand and consumption of poultry meat and poultry products are high, with human populations of 1 694 310 and 2 772 509 respectively (NBS, 2012). The selected districts in Mwanza were Ilemela, Nyamagana, Magu; whereas in Arusha, the districts were Arusha Urban, Arusha Rural, Meru, and Monduli.

3.2 Target population

3.2.1 Inclusion criteria

Chickens that were about to enter the food chain were sampled in this study. They included broilers that reached the slaughter weight and culled layer hens that had reached the end of their laying period and were being considered to be sold for human consumption (spent layers). The focus was on the commercial and semi-commercial broiler and layer production systems to capture resistance profiles in these different systems.

3.2.2 Exclusion criteria

Sick chickens (broilers and layers) were excluded from sampling, as they could not represent the status of resistance in bacteria carried by chickens that enter the food chain.

3.3 Selection of sampling areas

Farms were selected based on having more than 100 broilers and/or layer chickens kept for commercial purposes.

3.4 Sampling design

Sampling was conducted mainly directly from farms. Information was obtained regarding the farm; number of birds, last date of treatment, and the type of bird. The sampling frame was composed of broiler and layer chicken farms; the sampling units were broiler and layer chickens.

3.5 Sample size

The sample size was determined using infinite sample size calculation formula;

$$(n) = \frac{Z^2 Pq}{d^2}.$$

Where (n) is the required sample size, Z= Z value for a given confidence level, p = expected prevalence, q = (1 - p) and d = allowable error of estimation. The confidence level was assumed to be 95% with an allowable error of 5%, and thus Z is 1.96. Prevalence of 50% was used in the calculation, which resulted to n = 384 as the minimum sample size of the farms from where the samples were to be collected however, this number was raised to 402 farms to cover for any inconveniences which might arise during sample collection such as being denied a chance to sample from a farm that had consented before the exercise. Therefore 402 chicken cloaca swabs were collected from the farms.

3.6 Sample collection, packaging, labeling, transportation, and storage

The samples were collected from the cloaca of apparently healthy layer/broiler chickens using cotton wool sterile swabs. Each “sample-containing” tube was labeled with the sample identifier corresponding to the information on the sample identification (ID) sheet, location, and date. A sample collection form for each sample was filled with descriptive information that helped to correctly identify the sample, it contained information such as date of collection, sample ID, the farm name, and the region. The collected samples in leak-proof containers were stored in cool boxes with ice packs in the field while ensuring that there was no direct contact between the ice and the sample. The samples were then transported to the Microbiology laboratory at the College of Veterinary Medicine and Biomedical Sciences of the Sokoine University of Agriculture for analysis.

3.7 Isolation and identification of *Escherichia coli*

A loop full of the swab sample in a transport medium (maximum recovery diluent) was streaked on MacConkey agar (Oxoid Ltd., Basingstoke, UK), and incubated aerobically at 37ºC for 24 hours. The presumptive colonies obtained were sub-cultured on blood agar (Oxoid Ltd., Basingstoke, UK) at 37ºC for 24 hours aerobically. The pure colonies were then subjected to gram staining and biochemical tests i.e. Indole, Methyl red, Voges Proskauer, and Citrate utilization (IMViC, Sigma-Aldrich Co., Switzerland) according to the manufacturer’s instructions to identify *E. coli*. The identified pure isolates were preserved in Mueller Hinton Broth containing 15% v/v glycerol and stored at -80 ºC. The obtained *E. coli* isolates were further confirmed using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI TOF/MS) (Jackson and Lay, 2001).

3.8 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of bacterial isolates was performed in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018) adopting the disc diffusion method on Muller Hinton (MH) agar (Oxoid Ltd, Basingstoke, UK), as described by Luangtongkum *et al*. (2007). The preserved pure *E. coli* isolates were recuperated on MacConkey agar, purified in blood agar and nutrient agar (Oxoid Ltd., Basingstoke, UK) then subjected to sensitivity test. Three to five pure colonies were obtained and suspended in 2 ml of sterile normal saline and its turbidity was adjusted to that of the standard 0.5 McFarland (Oxoid Ltd., Basingstoke, UK). Thereafter, a sterile swab was dipped in the suspension and used to inoculate the *E. coli* suspension on Mueller Hinton agar (Oxoid Ltd., Basingstoke, UK). Afterward, the antimicrobial discs were placed on the dried inoculated Mueller Hinton agar plate using a q1BBL Sensi-disc dispenser (Fisher Scientific, UK) then incubated at 37°C for 18-24 hours. The inhibition zones were measured in millimeters using a ruler then the results were interpreted using the CLSI guidelines 2018 and also the manufacturer's instructions.

The isolates were documented as susceptible (S), intermediate (I), or resistant (R) according to clinical breakpoints defined by CLSI together with the zone diameters (mm). The most commonly used antimicrobial agents in livestock and humans in the study area and others recommended by WHO (2017) were tested for resistance; they included carbapenems (Ertapenem 10µg S≥22, I=20-22, R≤19), phenicols (Chloramphenicol 30 µg S≥18, I=13-17, R≤12), cephems (ceftriaxone 30 µg: S≥23, I=20-22, R≤19), quinolones and fluoroquinoles (Ciprofloxacin 5 µg: S≥21, I=16-20, R≤15), penicillin (Ampicillin 10 µg): S≥17, I=14-16, R≤13, folates (Trimethoprim/ Sulfamethoxazole 25 µg: S≥16, I=11-15, R≤10) and Aminoglycosides (Gentamicin 120 µg: S≥15, I=13-14, R≤12). *E. coli* ATCC 25922 was used as a reference strain and for

quality control purposes. The prevalence of isolates that were resistant to any number of antimicrobial agents was reported. An isolate that was resistant to three or more classes of antimicrobials was referred to as multi-drug resistant as defined by Magiorakos *et al*. (2012).

3.9 Phenotypic and molecular determination of ESBL producing *E. coli*

3.9.1 Phenotypic determination of ESBL producing *E. coli*

The *E. coli* isolates that produced ESBL enzymes were determined using the double-disc synergy method using cefotaxime (CTX-30 µg) and cefotaxime/clavulanic acid (CTX/ CLA-30 µg/10 µg) and ceftazidime (30 µg) alone and ceftazidime /clavulanic acid (10µg) as recommended by CLSI 2018 using disk diffusion method. A single pure colony was obtained from nutrient agar and suspended in 2ml of sterile normal saline and its turbidity was brought up to that of the standard 0.5 McFarland (Oxoid Ltd., Basingstoke, UK). Thereafter, a sterile swab was dipped in the suspension and used to inoculate the *E. coli* suspension on Mueller Hinton agar (Oxoid Ltd., Basingstoke, UK) by thoroughly/ completely streaking the plate. Afterward, the antimicrobial discs were placed on the inoculated Mueller Hinton agar plate using a q1BBL Sensi-disc dispenser (Fisher Scientific, UK) then incubated at 37°C for 24hours. The zones of inhibition were measured in millimeters using a ruler then the results were interpreted using the CLSI guidelines 2018 and also the manufacturer's guidelines. A difference in distance of ≥5mm increase in zone of diameter was termed as indicating ESBL producing *E. coli*.

3.9.2 Molecular determination of ESBL producing *Escherichia coli*

3.9.2.1 DNA extraction

The isolates that were found phenotypically ESBL positive were sub-cultured on MacConkey (Oxoid Ltd., Basingstoke, UK) and Nutrient agar (Oxoid Ltd., Basingstoke, UK), then 2-3 pure colonies of *E. coli* were picked and added in an Eppendorf tube containing 100 microliters of distilled water. The DNA was extracted by boiling method as previously described Malato *et al*. (2009) at 100ºC for 10 minutes. The Eppendorf tubes containing the mixture were suspended in boiling water for 10 minutes, then afterward they were removed and cooled in a freezer for 5 minutes then re-boiled for 10 minutes and afterward cooled for 5 minutes in a freezer. The DNA was extracted by centrifuging at the highest speed for 3 minutes.

3.9.2.2 Preparation of working primers for *blaSHV* and *blaCTX-M*

The master mix was prepared for all the phenotypic ESBL positive samples by mixing PCR premix (12.5µL), forward primer for bla_{SHV} and $bla_{CTX\text{-}M}$ (0.5 µL each), a reverse primer for bla_{SHV} and $bla_{CTX\text{-}M}$ (0.5 µL) (Table 3.1), and then 2.5 µL of nuclease-free water were prepared and brought up to the volume of 21 ESBL samples. Then it was aliquoted to the PCR Eppendorf tubes and thereafter 3 µL of each extracted DNA was added to the PCR tubes containing the master mix. ESBL $bla_{CTX\text{-}M}$ and bla_{SHV} were identified using multiplex PCR with the following PCR amplification conditions: Initial denaturation at 95ºC for 5minutes, then denatured for 30 cycles at 94 ºC for 30 seconds, annealing was done at 58 ºC for 30 seconds, the extension was performed at 72 ºC for 2 minutes then the final extension was set at 72 ºC for 10minutes (Woodford *et al*., 2005; Tofeland *et al*., 2007; Malato *et al*., 2009).

3.9.2.3 Molecular identification of *blaTEM*

The master mix was prepared for all the phenotypic ESBL positive samples by mixing PCR premix (12.5µL), forward primer for bla_{TEM} (0.5 µL each), a reverse primer for bla_{TEM} (0.5 µL) (Table 3.1), and then 3.5 µL of nuclease-free water was prepared and brought up to the volume of 21 samples. Then it was aliquoted to the PCR tubes and thereafter 3 µL of each extracted DNA was added to the PCR tubes containing the master mix. ESBL bla_{TEM} genes were identified using uniplex PCR with the following PCR amplification conditions: Initial denaturation at 95ºC for 5minutes, then denatured for 30cycles at 94 ºC for 15 seconds, annealing was done at 58 ºC for 30 seconds, the extension was performed at 72 ºC for 2 minutes then the final extension was set at 72 ºC for 10minutes (Tofeland *et al*., 2007)

Table 3.1: Target gene and PCR primers

Antimicrobial class	Gene	Primer (5' -3')	Size	A/temp	Ref
Cephalosporins	$\boldsymbol{bla_{TEM}}$	F-ATG AGT ATT CAA CAT TTC CG R-CCA ATG CTT AAT CAG TGA GG	858	50	Tofeland *et al* (2007)
	$\boldsymbol{bla_{SHV}}$	F-ATG CGT TAT ATT CGC CTG TG R-AGC GTT GCC AGT GCT CGA TC	862	58	Tofeland *et al* (2007)
	Universal $\boldsymbol{bla_{CTX-M}}$	F-5′-SCS ATG TGC AGY ACC AGT AA R-5′-CCG CRA TAT GRT TGG TG	554	58	Woodford *et al*. (2005) Malato *et al*. (2009)

3.9.2.4 Gel electrophoresis preparation

Working buffer was prepared by mixing 20 mls of TAE buffer stock solution and 980 ml of distilled water in a conical flask. Afterward, 1% agarose gel was prepared by weighing 1 gram of agarose which was dissolved in 100mls of the working TAE buffer. The working TAE buffer was poured into the gel tank containing combs, wells were made and the gene bands were visualized using 2.5 µL of ethidium bromide dye, and the gel image

was documented using Biobase PCR Gel Document imaging System for DNA. Those that produced bands were termed positive whereas those that didn't show bands were termed as negative.

3.10 MALDI-TOF MS confirmation of *E. coli*

The ethanol extraction procedure was carefully performed as per the manufacturer's instruction (Bruker Daltonik GmbH, Bremen. Germany). Two to three pure colonies of each (402 isolates) *E. coli* were added in a screw cap extraction tube containing 300 µL of double-distilled water. 0.9ml of absolute ethanol was added to the tube and shaken well to mix the content; thereafter the tubes were centrifuged at 13 000 rpm for two minutes. The pellets obtained after pouring the supernatant were air-dried. 10 µL of the pellets were mixed with 50 µL of 70% formic acid and acetonitrile (same amount as of the mixture of the pellets and formic acid) was added. The mixture was then centrifuged at 13 000 rpm for two minutes, afterward, 1 µL of the supernatant obtained was poured on the ground steel MALD target plate and air left to air dry at room temperature. Then, each sample was overlaid with 1 µL of matrix solution (saturated solution of α-cyano-4 hydroxy-cinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) and then air-dried at room temperature. The MALDI TOF MS target was subsequently introduced into the MALDI-TOF MS (Bruker Daltonik GmbH, Bremen. Germany) for automated measurement and data interpretation. All samples were blinded and run in duplicates. ATCC 25922 *E coli* strain was used for reference. The samples were analyzed using a Microflex LT MALDI-TOF MS instrument (Bruker Daltonik GmbH, Bremen. Germany). The spectra were recorded in the linear positive mode at a laser frequency of 20HZ within a mass range from 2,000 to 20,000Da. The raw spectra were processed using the MALDI BioTyper 1.1 software (Bruker Daltonik GmbH, Bremen. Germany) with default settings. The highest log score of a match against the score in the database was used for species identification (Jackson and Lay, 2001; Panda *et al*., 2014).

3.11 Data analysis

Data were captured in Microsoft excel® and analyzed using SPSS version 20. Statistical significance was set at p-value <0.05 and the prevalence for *E. coli* from broilers and layers were determined using a large contingency chi-square.

CHAPTER FOUR

4.0 RESULTS

4.1 Overall prevalence of *E. coli* in Mwanza and Arusha regions

E. coli isolates were isolated from all the 402 (100%) samples. Random selection of the isolated *E. coli* was performed and 204 (50.7%) isolates were chosen and then subjected to an antimicrobial sensitivity test. Out of 204 selected isolates, 103 (50.5%) were from broilers and 101(49.5%) were from layers chickens. The randomly chosen isolates from the Arusha region were 102 (50%) and 102 (50%) were from the Mwanza region in Tanzania. Twenty-one isolates tested positive for ESBL phenotypically and showed the presence of bla_{TEM} genes and only two isolates (2/21) had $bla_{CTX\text{-}M}$. None of the isolates expressed the bla_{SHV} gene in this study (Table 4.14).

Overall, the highest resistance was observed for ampicillin (100%), whereas the lowest prevalence of resistance (10.3%) was recorded for gentamicin. Frequencies of resistance against other antimicrobial agents are as presented in Table 4.1. There was no significant variation of antimicrobial resistance by chicken type (broiler and layer) against the tested selected antimicrobial agents as shown in Table 4.2.

Table 4.1: Overall antimicrobial resistance prevalence of randomly selected *E. coli* isolates to selected antimicrobial agents in Arusha and Mwanza

S/no	Antimicrobial agent	Frequency	%
1	Ertapenem	62	30.4
2	Chloramphenicol	110	53.9
3	Ceftriaxone	95	46.6
4	Ciprofloxacin	140	68.6
5	Ampicillin	204	100
6	Trimethoprim/ Sulfamethoxazole	182	89.2
7	Gentamicin	21	10.3

Table 4.2: Prevalence of resistance to selected antimicrobial agents among *E. coli* isolates obtained from broiler and layer chickens in Arusha and Mwanza by chicken type

S/N	Antimicrobial agent	The prevalence of resistance n (%)		P. value
		Broiler (n=103)	Layer (n=101)	
1	Ertapenem	32 (15.7)	30(14.7)	0.832
2	Chloramphenicol	58 (28.4)	52 (25.5)	0.489
3	Ceftriaxone	53 (26.0)	21 (20.6)	0.158
4	Ciprofloxacin	71 (34.8)	69(33.8)	0.925
5	Ampicillin	103 (50.5)	101 (49.5)	_
6	Trimethoprim/	96 (47.1)	86 (42.2)	0.064
7	Gentamycin	13(6.4)	8(3.9)	0.269

The highest resistance to ertapenem drug was recorded in the Ilemela district while the Nyamagana district recorded the highest prevalence of resistance to chloramphenicol (14.7%), ceftriaxone (11.8%), and ampicillin (24.5%). The highest resistance to ciprofloxacin (21.6%) and gentamycin (3.4%) was recorded in the Ilemela district. Trimethoprim/ Sulfamethoxazole drug indicated the lowest prevalence in the Magu district (Figure 4.1).

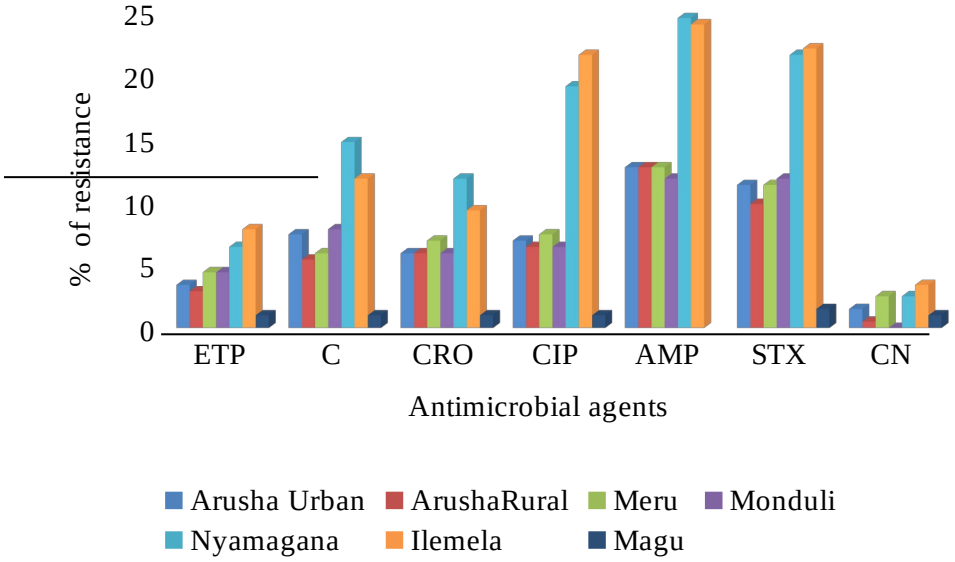

Figure 4.1: Prevalence of resistance to selected antimicrobial agents among *E. coli* isolates obtained from broiler and layer chickens in Arusha and Mwanza by the district.

There was a statistically significant variation in antimicrobial resistance by the district against ciprofloxacin (p=0.001). Only 27/204 *E. coli* isolates (13.24%) did not exhibit multidrug resistance patterns. Of these, 5.4% showed resistance to only one class of antimicrobial agents whereas 7.8% depicted resistance to at most two classes of antimicrobial agents (Figure 4. 2).

One hundred and seventy-seven (86.76%) selected *E. coli* isolates showed resistance to more than two classes of the tested antimicrobials. Resistance to four classes of antimicrobial agents was the highest with a prevalence of 31.1%, followed by resistance to 3 classes of antimicrobial agents (28.2%). Resistance to 7 classes was the lowest (3.4%) as shown in Figure 4.2 and Table 4.3 below.

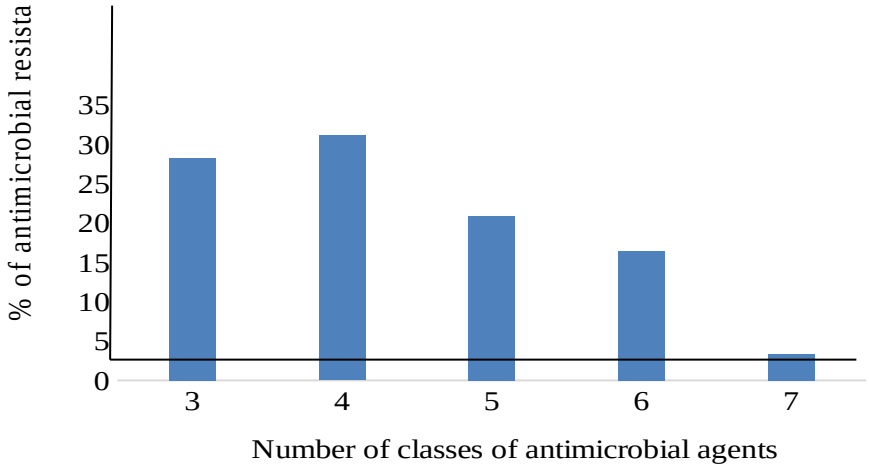

Figure 4.2 Multidrug resistance among 204 randomly selected *E. coli* isolated from broiler and layer chickens from Arusha and Mwanza

The most common pattern of multidrug resistance was observed in 28 selected *E. coli* isolates in both Mwanza and Arusha (ETP, C, CRO, CIP, AMP, STX) which means that the E. *coli* isolates were resistant to six classes of antimicrobial agents, followed by (C, CIP, AMP, STX) where 20 isolates depicted similar resistance to four classes of the selected antimicrobial agents and the third most observed resistance pattern was (CRO, CIP, AMP) where the *E. coli* were resistant to three classes of antimicrobial agents with a similar pattern as seen in Table 4.3.

Table 4.3: Multidrug resistance patterns of *E. coli* isolates from broiler and layer chickens from Arusha and Mwanza

Antimicrobial agents combination	Number of isolates	Percentage	Number of antimicrobial classes
C,CIP,AMP	2	1.13	3
C,AMP,STX	16	9.04	3
CIP,AMP,CN	1	0.56	3
ETP,AMP,STX	1	0.56	3
CIP,AMP,STX	20	11.3	3
CRO,CIP,AMP	1	0.56	3
CRO,AMP,STX	9	5.08	3
C,CIP,AMP,STX	24	13.56	4
ETP,C,AMP,STX	1	0.56	4
C,CRO,AMP,STX	5	2.82	4
CIP,AMP,STX,CN	1	0.56	4
ETP,CIP,AMP,STX	3	1.69	4
CRO,CIP,AMP,STX	14	7.91	4
ETP,CRO,CIP,AMP	2	1.13	4
ETP,CRO,AMP,STX	5	2.82	4
C,CIP,AMP,STX,CN	5	2.82	5
ETP,C,CIP,AMP,STX	8	4.52	5
ETP,C,CRO,CIP,AMP	1	0.56	5
C,CRO,AMP,STX,CN	1	0.56	5
C,CRO,CIP,AMP,STX	13	7.34	5
CRO,CIP,AMP,STX,CN	2	1.13	5
ETP,CRO,AMP,STX,CN	1	0.56	5
ETP,CRO,CIP,AMP,STX	6	3.39	5
ETP,C,CRO,CIP,AMP,STX	28	15.82	6
ETP,CRO,CIP,AMP,STX,CN	1	0.56	6
ETP,C,CRO,CIP,AMP,STX,CN	6	3.39	7

4.2 Prevalence of antimicrobial resistance of selected *E. coli* isolates in Mwanza region

Fifty-two selected *E. coli* isolates from the Mwanza region were from broilers whereas 50 isolates were from layers. The most prevalent resistance of *E. coli* was observed for Ampicillin (100%), followed by Trimethoprim/Sulfamethoxazole (90.2%) and ciprofloxacin (83.3%) whereas the lowest prevalence of antimicrobial resistance was observed for Gentamycin (11.8%) (Table 4.4).

Table 4.4: Prevalence of resistance to selected antimicrobial agents among *E. coli* isolated from broiler and layer chickens from Mwanza

Sn	Antimicrobial agent	Number of isolates	Percentage
1	Ertapenem	31	30.4
2	Chloramphenicol	56	54.9
3	Ceftriaxone	45	44.1
4	Ciprofloxacin	85	83.3
5	Ampicillin	102	100
6	Trimethoprim/ Sulfamethoxazole	92	90.2
7	Gentamycin	12	11.8

E. coli isolates obtained from broiler chickens showed higher resistance to Ertapenem (15.7%), Ceftriaxone (23.5%), Ampicillin (51.0%), Trimethoprim/ Sulfamethoxazole (47.1%), and Gentamycin (6.9%) compared to *E. coli* isolated from layers in Mwanza region. Higher prevalence of antimicrobial resistance were observed in layers against chloramphenicol (28.4%) and ciprofloxacin (42.2%) than broiler *E. coli* isolates. Differences in antimicrobial resistance to any of the selected antimicrobial agents by chicken type (broiler and layer) were not statistically significant (Table 4.5).

Table 4.5: Prevalence of antimicrobial resistance by chicken type

Sn	Antimicrobial agents	Broiler n (%)	Layer n (%)	P. value
1	Ertapenem	16(15.7)	15(14.7)	0.933
2	Chloramphenicol	27(26.5)	29(28.4)	0.538
3	Ceftriaxone	24(23.5)	7(6.9)	0.673
4	Ciprofloxacin	42(41.0)	43(42.2)	0.479
5	Ampicillin	52(51.0)	50(49)	-
6	Trimethoprim/ sulfamethoxazole	48(47.1)	44(43.1)	0.465
7	Gentamycin	7(6.9)	5(4.9)	0.588

E. coli isolates obtained from samples collected in the Ilemela district recorded the highest antimicrobial resistance against Trimethoprim/Sulfamethoxazole in broilers (22.5%) and Ertapenem in layers (8.8%). Similarly, a high antimicrobial resistance prevalence was observed in broiler and layer chickens (14.7%) against Chloramphenicol

in the Nyamagana district. Resistance against Ceftriaxone was highest among *E. coli* isolates obtained from broilers in Nyamagana district in Mwanza Region. In the Ilemela district, *E. coli* isolates obtained from both broiler and layer chickens recorded a similar and the highest antimicrobial resistance (Table 4.6). Only 9.8% (10/102) of the *E. coli* isolates didn't show a multidrug resistance pattern in the Mwanza region. Six isolates were resistant to at most two classes of the selected antimicrobial agents in this study, whereas four *E. coli* isolates showed resistance to only one class of the selected antimicrobials. The *E. coli* isolates that showed multidrug resistance in the Mwanza region were 92/102 with 34.8% being resistant to four classes of the antimicrobial agents used in this study. Five *E. coli* isolates (5.3%) were resistant to all the seven classes of antimicrobial agents used in this study (Table 4.7).

Table 4.6: Prevalence of antimicrobial resistance of randomly chosen *E. coli* isolates to selected antimicrobial agents by district and by chicken type

Sn	Antimicrobial agent	Chicken type	Ilemela (%)	Magu (%)	Nyamagana (%)	P
1	Ertapenem	Broiler	6.9	2	6.9	0.38
		Layer	8.8	0	5.9	0.355
2	Chloramphenicol	Broiler	9.8	2	14.7	0.382
		Layer	13.7	0	14.7	0.774
3	Ceftriaxone	Broiler	8.8	2	12.7	0.455
		Layer	9.8	0	10.8	0.774
4	Ciprofloxacin	Broiler	21.6	2	17.6	0.178
		Layer	21.6	0	20.6	0.684
5	Ampicillin	Broiler	23.5	2	24.5	
		Layer	24.5	0	24.5	
6	Trimethoprim/sulfamethoxazole	Broiler	22.5	2.9	21.6	0.516
		Layer	21.6	0	21.6	1
7	Gentamycin	Broiler	3.9	2.9	2.9	0.696
		Layer	2.9	0	2	0.637

Table 4.7: Prevalence of multidrug resistance among *E. coli* isolates obtained from broiler and layer chickens in Tanzania

Number of classes of antimicrobial agent	Number of isolates	Percent
3	22	23.9
4	32	34.8
5	20	21.7
6	13	14.1

7	5	5.3

The predominant pattern of antimicrobial resistance was observed among fifteen *E. coli* isolates which showed multidrug resistance to four classes of antimicrobial agents used in this study (C, CIP, AMP, STX), followed by antimicrobial resistance to three classes of antimicrobial agents (CIP, AMP, STX). Five isolates showed resistance to all the seven classes of antimicrobial agents used in this study (Table 4.8).

Table 4.8: Multidrug resistance patterns of *E. coli* obtained from broiler and layer chickens in Mwanza

Drugs resistance pattern	Antimicrobial agent classes	Number of isolates	Percentage
C,CIP,AMP	3	1	1.09
C,AMP,STX	3	3	3.26
CIP,AMP,STX	3	14	15.22
CRO,CIP,AMP	3	2	2.17
ETP,AMP,STX	3	1	1.09
CRO,AMP,STX	3	1	1.09
C,CIP,AMP,STX	4	15	16.30
ETP,C,AMP,STX	4	1	1.09
C,CRO,AMP,STX	4	1	1.09
CIP,AMP,STX,CN	4	1	1.09
ETP,CIP,AMP,STX	4	3	3.26
ETP,CRO,AMP,STX	4	1	1.09
CRO,CIP,AMP,STX	4	10	10.87
C,CIP,AMP,STX,CN	5	3	3.26
ETP,C,CIP,AMP,STX	5	5	5.43
ETP,C,CRO,AMP,CIP	5	1	1.09
C,CRO,CIP,AMP,STX	5	7	7.61
CRO,CIP,AMP,STX,CN	5	1	1.09
ETP,CRO,CIP,AMP,STX	5	3	3.26
C,CRO,CIP,AMP,STX,CN	6	1	1.09
ETP,C,CRO,CIP,AMP,CN	6	1	1.09
ETP,C,CRO,CIP,AMP,STX	6	11	11.96
ETP,C,CRO,CIP,AMP,STX,CN	7	5	5.43

4.3 Prevalence of antimicrobial resistance of selected *E. coli* isolates in Arusha region, Tanzania

E. coli isolates obtained from both broiler and layer chickens in the Arusha region had the same prevalence of 25.5% with exception of the Monduli district (Table 4.9). In Arusha, 100% antimicrobial resistance of the selected *E. coli* isolates was observed in ampicillin, followed by 88.3% antimicrobial resistance to Trimethoprim/ Sulfamethoxazole, with the least prevalence of antimicrobial resistance observed for gentamicin (8.8%) (Table 4.10). Variation of resistance in selected *E. coli* isolates against chloramphenicol by chicken type and by district was statistically significant in Arusha (p=0.049) (Table 4.11).

Table 4.9: Frequency (%) of antimicrobial resistant *E. coli* isolates from broiler and layer chickens in Arusha (Feb, 2021)

District	*E. coli* isolates frequency (%)		Confidence interval	
	Broiler (n=51)	Layer (n=51)	Lower	Upper
Arusha rural (n=26)	25.5	25.5	17.6	33.3
Arusha urban(n=26)	25.5	25.5	16.7	34.3
Meru (n=26)	25.5	25.5	17.6	34.3
Monduli (n=24)	23.5	23.5	15.7	31.4

Table 4.10: Antimicrobial resistance to selected antimicrobial agents among isolates obtained from broiler and layer chickens in Arusha by chicken type

Sn	Antimicrobial agent	Prevalence of resistance n (%)		Overall prevalence (%)	P. value
		Broiler	Layer		
1	Ertapenem	16 (15.7)	15 (14.7)	30.4	0.830
2	Chloramphenicol	31 (30.4)	23 (22.5)	52.9	0.113
3	Ceftriaxone	29 (28.4)	21 (20.6)	49	0.113
4	Ciprofloxacin	29 (28.4)	26 (25.5)	53.9	0.551
5	Ampicillin	51 (50)	51 (50)	100	_
6	Trimethoprim/ Sulfamethoxazole	48 (47.1)	42 (41.2)	88.3	0.065
7	Gentamycin	6 (5.9)	3 (2.9)	8.8	0.295

Table 4.11: Prevalence of resistance to selected antimicrobial agents among *E. coli* isolates obtained from broiler and layer chickens in Arusha by chicken type and by district

Sn	Agent	Type	Arusha rural	Arusha urban	Meru	Monduli	P. value
1	Ertapenem	B	4(3.9)	4(3.9)	4(3.9)	4(3.9)	0.999
		L	2(2.0)	3(2.9)	5(4.9)	5(4.9)	0.413
2	Chloramphenicol	B	8(7.8)	9(8.8)	4(3.9)	10(9.8)	0.049
		L	3(2.9)	6(5.9)	8(7.8)	6(5.9)	0.252
3	Ceftriaxone	B	7(6.9)	8(7.8)	7(6.9)	7(6.9)	0.974
		L	5(4.9)	4(3.9)	7(6.9)	5(4.9)	0.686
4	Ciprofloxacin	B	7(6.9)	7(6.9)	7(6.9)	8(7.8)	0.893
		L	6(5.9)	7(6.9)	8(7.8)	5(4.9)	0.763
5	Ampicillin	B	13(12.7)	13(12.7)	13(12.7)	12(11.8)	_
		L	13(12.7)	13(12.7)	13(12.7)	12(11.8)	_
6	Trimethoprim/ Sulfamethoxazole	B	12(11.8)	12(11.8)	12(11.8)	12(11.8)	0.806
		L	8(7.8)	11(10.8)	11(10.8)	12(11.8)	0.088
7	Gentamycin	B	1(1.0)	2(2.0)	3(2.9)	0(0.0)	0.311
		L	0(0.0)	1(1.0)	2(2.0)	0(0.0)	0.289

Key: B=Broilers and L=Layers

Twenty-eight *E. coli* isolates obtained from the Arusha region were resistant to three classes of antimicrobial agents tested under this study with 1.2% of the isolates showing antimicrobial resistance to all the seven antimicrobial agents. Twenty-three (27.1%) of the isolates were resistant to four classes of the antimicrobial agents, 20.0% showed antimicrobial resistance to five classes of antimicrobial agents and 18.8% were resistant to six classes of antimicrobial agent tested in this study.

The most common observed multidrug resistance pattern among *E. coli* isolates in this study was seen in six classes of antimicrobials (ETP, C, CRO, CIP, AMP, STX) (Table 4.12).

Table 4.12: Patterns of multidrug resistance of selected *E. coli* isolated from broiler and layer chickens in Arusha

Antimicrobial combination	Number of isolates	Percentage	Classes of antimicrobial agents
C,AMP,STX	13	15.3	3
CIP,AMP,CN	1	1.2	3
CIP,AMP,STX	6	7.1	3
CRO,AMP,STX	8	9.4	3
C,CIP,AMP,STX	10	11.8	4
C,CRO,AMP,STX	4	4.7	4
ETP,CIP,AMP,STX	1	1.2	4
ETP,CRO,CIP,AMP	2	2.4	4
CRO,CIP,AMP,STX	3	3.5	4
ETP,CRO,AMP,STX	3	3.5	4
C,CIP,AMP,STX,CN	2	2.4	5
ETP,C,CIP,AMP,STX	3	3.5	5
C,CRO,AMP,STX,CN	1	1.2	5
C,CRO,CIP,AMP,STX	5	5.9	5
CRO,CIP,AMP,STX,CN	1	1.2	5
ETP,CRO,CIP,AMP,STX	4	4.7	5
ETP,CRO,AMP,STX,CN	1	1.2	6
ETP,C,CRO,AMP,STX,CN	1	1.2	6
ETP,C,CRO,CIP,AMP,STX	14	16.5	6
ETP,CRO,CIP,AMP,STX,CN	1	1.2	6
ETP,C,CRO,CIP,AMP,STX,CN	1	1.2	7

4.4 ESBL producing *E. coli* isolates from broiler and layer chicken in Arusha and Mwanza, Tanzania

The overall prevalence of ESBL producing *E. coli* was 10.29%. All ESBL producing *E. coli* isolates were 100% resistant to both ampicillin and Trimethoprim/ Sulfamethoxazole but had the lowest resistance to gentamycin (Table 4.13). Broilers showed a higher prevalence of resistance than layers to all antimicrobials except for Ertapenem (Table 4.14).

Table 4.13: Antimicrobial resistance of ESBL producing *E. coli* isolated from broiler and layer chicken in Arusha and Mwanza, Tanzania

Antimicrobial agents	Number of isolates	Percentage
Ertapenem	5	23.8
Chloramphenicol	9	42.9
Ceftriaxone	16	76.2
Ciprofloxacin	12	57.1
Ampicillin	21	100
Trimethoprim/Sulfamethoxazole	21	100
Gentamycin	3	14.3

The most prevalent extended spectrum beta-lactamase gene in this study was bla_{TEM} whereas the bla_{SHV} gene was not detected in all isolates. Samples from Arusha had a higher number of ESBL *E. coli* isolates compared to Mwanza with bla_{TEM} being more prevalent as shown in Table 4.14.

Table 4.14: Overall prevalence of ESBL producing *E. coli* obtained from Mwanza and Arusha regions in Tanzania

Antimicrobial gene	Broiler	Layer	Arusha	Mwanza	Total
bla_{TEM}	13 (61.9)	8(38.1)	17(81.0)	4(19.0)	100
$bla_{CTX\text{-}M}$	0(0.0)	2(9.5)	2(9.5)	0(0.0)	9.5
bla_{SHV}	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0

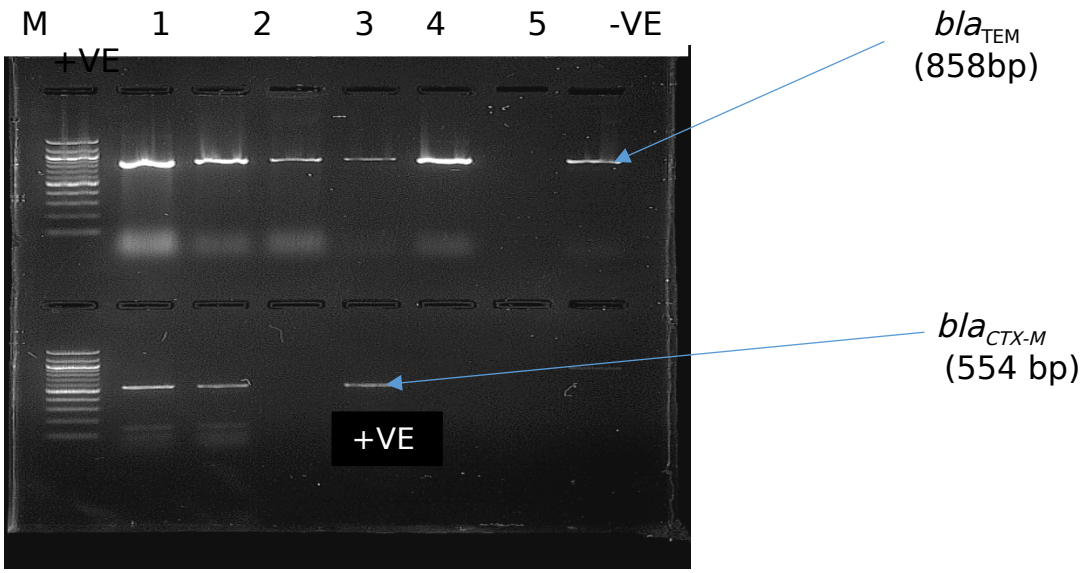

Plate 4.1: Amplified *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes of five out of 21 ESBL positive samples

Note: Samples 1 and 2 express both ESBL genes and samples 3-5 have expressed the gene for *bla*$_{TEM}$. M=Marker 100bp DNA ladder, 1-5= samples, -VE=negative control, +VE=positive control, bp=base pair.

Twenty-three-point eight one percent (23.81%) of the ESBL producing *E. coli* isolates observed in this study were resistant to three classes of antimicrobial agents with the following antimicrobial agent combination (CRO, AMP, STX). Only one (4.76%) of the ESBL producing *E. coli* was resistant to six classes of the antimicrobial agents tested in this study (Table 4.15).

Table 4.15: Multidrug resistance pattern of the ESBL positive *E. coli*

Antimicrobials agents combination	Number of Antimicrobial agent classes	Number of isolates	%
AMP, STX, CN	3	1	4.76
CIP, AMP, STX	3	2	9.52
CRO, AMP, STX	3	5	23.81
C,CRO,AMP, STX	4	2	9.52
ETP, CRO,AMP, STX	4	1	4.76
C, CIP, AMP, STX, CN	5	1	4.76
C, CRO, CIP, AMP,STX	5	4	19.05
ETP, C, CIP, AMP, STX	5	1	4.76
CRO, CIP, AMP, STX, CN	5	1	4.76
ETP, CRO, CIP, AMP, STX	5	2	9.52
ETP,C, CRO, CIP,AMP, STX	6	1	4.76

CHAPTER FIVE

5.0 DISCUSSION

Antimicrobial resistance was observed in all the selected *E. coli* isolates in this study with every isolate being resistant to at least one of the antimicrobial agents used in this study. This observation differed from that of a study carried out in Nairobi, Kenya where all the *E. coli* isolates (n=85) were susceptible to gentamicin and ciprofloxacin (Langata *et al.*, 2019). This high resistance of *E. coli* to all antimicrobial agents tested might have occurred as a result of continuous uncontrolled overuse and misuse of antimicrobial agents in poultry farms, which was observed by Hounmanou and Mdegela (2017); Katakweba *et al.* (2018); Kimera *et al*. (2020).

The overall prevalence of multidrug resistance among selected *E. coli* isolates according to this study was 86.76% (177/204) in both Mwanza and Arusha which was significantly higher compared to a study conducted in Dar es salaam where the prevalence of MDR *E. coli* of broilers and layers was 69.3% (147/212) (Mgaya *et al*., 2021) and 51.6% MDR as reported by Kimera *et al*. (2021). This prevalence of MDR *E. coli* isolates is also higher than that of *E. coli* isolates obtained from local and imported retail chicken carcasses in Qatar which was 63.4% (Eltai *et al*., 2020), also higher than a prevalence of MDR *E. coli* isolates of 68% obtained from healthy chicken farms in the region of Dakar, Senegal (Vounba *et al*., 2019). However, Rahman *et al*. (2020) reported a slightly lower prevalence of MDR *E. coli* isolates obtained from broiler and layer chickens which was 49.23% for broilers and 51.09% for layer chicken in Sylhet division, Bangladesh. Muha *et al*. (2019) reported 100% MDR of *E. coli* isolated from cloaca swabs of live broilers which were significantly higher compared to the MDR findings of this study.

The *E. coli* isolates obtained in this study showed different prevalence of resistance to the different antimicrobial agents. The highest resistance was observed for ampicillin (100%) which is comparable to previous reports (Igizeneza, 2020; Sarker *et al*., 2019; Roedel *et al*., 2021; Seo and Lee, 2021). A high prevalence of antimicrobial resistance to ampicillin was also observed in *E. coli* isolated from frozen chicken meat in Bangladesh by Parvin *et al*. (2020). A similar trend of AMR was observed by Rugumisa *et al*. (2016) in Arusha District where the highest percentage of resistance was observed for Sulfamethoxazole (80.3%); though for ampicillin, the prevalence in this study significantly differs with their findings (49.1%) which most probably could mean that the overuse and misuse of ampicillin have increased in Arusha region. This high prevalence of antimicrobial resistance to ampicillin could result from the lower price of ampicillin and its easy availability where the antimicrobials are sold over the counter and administered to the chickens without any prescription by veterinarians which agrees with earlier studies (Aworth *et al*., 2020; Baran *et al*., 2020; Musa *et al*., 2020). In addition, the high antimicrobial resistance of *E. coli* in this study could be attributed to AmpC beta-lactamase which hinders the efficacy of beta-lactams in *E. coli* as previous reported (Pitout *et al*., 2007; Perez-Gallego *et al*., 2016) though these genes were not assessed in this study.

In the current study, the prevalence of resistance to trimethoprim /sulfamethoxazole (89.2%) was slightly lower compared to the findings of Sarker *et al*. (2019) where resistance was 94.59%. The resistance to STX (trimethoprim/sulfamethoxazole) observed in this study was significantly higher than that observed in backyard chicken in Ethiopia (Sarba *et al*., 2019), where the *E. coli* isolates were not resistant to STX.

Antimicrobial resistance to ciprofloxacin was also significantly higher (68.6%) which is

contrary to findings by Sarba *et al*. (2019) where the *E. coli* isolates showed no resistance to ciprofloxacin. An opposite trend of resistance was observed by Subramanya *et al*. (2020) and Musa *et al*. (2020) where 31.8% and 44.4% of *E. coli* were resistant to ciprofloxacin, respectively. Sana *et al*. (2021) recorded a higher resistance of *E. coli* to ciprofloxacin (74.4%) which differs from the findings of this research.

Antimicrobial resistance of *E. coli* to chloramphenicol had a prevalence of 53.9% which was slightly lower than 56.2% reported by Baran *et al*. (2020) in Eastern Turkey. Antimicrobial resistance to chloramphenicol might have occurred as a result of the close proximity of the chickens with humans especially where the hygienic practices are very low (Kimera *et al.,* 2020). The continuous interaction could have been a very important aspect for the increase in MDR in *E. coli* to antimicrobial agents that are commonly used by humans which could easily spill into the environment (Prescott, 2008; Huijbers *et al*., 2014).

In this study, antimicrobial resistance of *E. coli* to Ertapenem was 30.4% which differs from a study carried in chicken meat in Eastern Turkey where resistance to carbapenems was not observed (Baran *et al*., 2020). A high susceptibility of *E. coli* isolates to gentamicin was observed in this study which doesn't augment with Baran *et al*. (2020) and Aworth *et al*. (2020). The lower resistance to aminoglycosides was also observed in studies carried out by Abbassi *et al*. (2017) and Seo and Lee, (2020). The use of antimicrobial agents uncontrollably in broiler and layer chickens might have contributed to the multidrug resistance of *E. coli* which was observed in this study as observed in Tanzania by Caudell *et al*., 2018; Kimera *et al*., 2020).

The prevalence of ESBL producing *E. coli* isolates in this study was 10.29%, with 100%

possessing *bla*$_{\text{TEM}}$, 9.52% *bla*$_{CTX\text{-}M,}$ and no observed isolate expressing *bla*$_{SHV}$ gene. The prevalence of ESBL producing *E. coli* in this study was lower than the findings by Chisumba *et al*. (2016) who reported 20.1% of ESBL *E. coli*. This prevalence was not in tandem with Kimera *et al*. (2021) (where 80% of the ESBL gene was *bla*$_{CTX\text{-}M}$ with no observed *bla*$_{\text{TEM}}$ and *bla*$_{SHV}$ ESBL genes) and Mgaya *et al*. (2021) (where 2/20 isolates had *bla*$_{CTX\text{-}M}$ with no detection of *bla*$_{\text{TEM}}$ and *bla*$_{SHV}$ genes). A similar high prevalence of *bla*$_{\text{TEM}}$ was observed in chicken droppings in Nairobi (46%) though significantly higher compared to this study (Langata *et al*., 2019). In this study, all the ESBL producers were multidrug-resistant.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study found out that *E. coli* isolated from apparently healthy broiler and layer chicken in Arusha and Mwanza regions were resistant to at least one antimicrobial agent used in this study. This can be attributed to the continuous use of excessive antimicrobial drugs for prophylaxis and chemotherapy in order to maximize profits on their poultry business. This study also found out that some *E. coli* isolated from broiler and layer chickens in the study regions were multidrug-resistant. This research found out that all ESBL producing *E. coli* isolated from broiler and layer chickens in Mwanza and Arusha regions were multidrug resistant. The study hypothesizes that the MDR *Escherichia coli* isolates could transfer resistant genes to other bacteria in the gut microbiome of the other broiler and layer chickens in the farms and at markets and possibly to the humans (final consumers) and probably to the environment.

6.2 Recommendations

The study recommends continued antimicrobial resistance surveillance in broiler and layer chickens, and at large to the poultry farms and markets including those reared in individual households. The farmers in the study area should avoid the administration of antimicrobial agents to healthy broiler and layer chickens, and they should be advised on better ways to maintain hygiene in their farms to prevent the introduction and spread of bacteria including those which carry antimicrobial resistant genes. The study recommends adherence to strict measures while administering antimicrobials in the study area in order to curb the spread of AMR genes. Further research can be conducted to investigate whether the same resistance genes found in *E. coli* isolates in this study are similar to those that can be isolated from the poultry farmers and from the immediate environment.

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